Customized FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY DOCKET NO.
P069/US00/BAS

U.S. APPLICATION NO.
(If known, see 37 CFR 1.5)
09/647,780

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR99/00807	07 April 1999	08 April 1998

TITLE OF INVENTION: NOVEL MEMBRANE-BOUND METALLOPROTEASE NEP II AND THE USE...

APPLICANT(S) FOR DO/EO/US: OUIMET et al.

Applicant herewith submits to the US Designated/Elected Office (DO/EO/US) the following items and other information:

___ 1. This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

X 2. This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 USC 371.

___ 3. This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 USC 371(b) and PCT Art. 22 and 39(1).

___ 4. A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

___ 5. A **copy** of the International Application as filed (35 U.S.C. 371 (c)(2))

___ a. is transmitted herewith (required only if not transmitted by the International Bureau).

___ b. has been transmitted by the International Bureau.

___ c. is not required, as the application was filed in the United States Receiving Office (RO/US).

X 6. A **translation** of the International Application into English (35 U.S.C. 371(c)(2)).

___ 7. Amendments to the claims of the International Appln. under PCT Article 19 (35 USC 371 (c)(3))

___ a. are transmitted herewith (required only if not transmitted by the International Bureau).

___ b. have been transmitted by the International Bureau.

___ c. have not been made; however, the time limit for making such amendments had NOT expired.

___ d. have not been made and will not be made.

___ 8. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

X 9. An **oath**-or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

___ 10. A translation of the annexes to the Int'l Prelim. Exam. Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

___ 11. An **Information Disclosure Statement** under 37 C.F.R. 1.97 and 1.98.

X 12. An **Assignment** document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

X 13. A **First preliminary amendment**.

___ A Second or subsequent preliminary amendment.

___ 14. A substitute specification.

___ 15. A change of power of attorney and/or address letter.

X 16. Other items or information:

X Statement under 37 CFR 1.821 including diskette

X A copy of the Notification of Missing Requirements under 35 U.S.C. 371.

X In the event that a petition for extension of time is required to be submitted herewith, and in the event that a separate petition does not accompany this response, applicant hereby petitions under 37 CFR 1.136(a) for an extension of time of as many months as are required to render this submission timely. Any fee is authorized in 17(c).

Date: 26 December 2000

LARSON & TAYLOR, PLC • 1199 North Fairfax St. • Suite 900 • Alexandria, VA 22314

Customized FORM PTO-1390 U.S. DEPARTMENT OF COMMERCE PATENT AND TRADEMARK OFFICE

TRANSMITTAL LETTER TO THE UNITED STATES DESIGNATED/ELECTED OFFICE (DO/EO/US) CONCERNING A FILING UNDER 35 U.S.C. 371

ATTORNEY DOCKET NO.	U.S. APPLICATION NO. (If known, see 37 CFR 1.5)
P06910US00/BAS	09/647780

INTERNATIONAL APPLICATION NO.	INTERNATIONAL FILING DATE	PRIORITY DATE CLAIMED
PCT/FR99/00807	07 APRIL 1999	08 APRIL 1998

TITLE OF INVENTION: NOVEL NEP II MEMBRANE METALLOPROTEASE AND ITS USE FOR SCREENING. . .

APPLICANT(S) FOR DO/EO/US: OUIMET, Tanja et al.

Applicant herewith submits to the US Designated/Elected Office (DO/EO/US) the following items and other information:

☒ 1. This is a **FIRST** submission of items concerning a filing under 35 U.S.C. 371.

☐ 2. This is a **SECOND** or **SUBSEQUENT** submission of items concerning a filing under 35 USC 371.

☒ 3. This express request to begin national examination procedures (35 USC 371(f)) at any time rather than delay examination until the expiration of the applicable time limit set in 35 USC 371(b) and PCT Art. 22 and 39(1).

☒ 4. A proper Demand for International Preliminary Examination was made by the 19th month from the earliest claimed priority date.

☒ 5. A **copy** of the International Application as filed (35 U.S.C. 371 (c)(2))

- ☐ a. is transmitted herewith (required only if not transmitted by the International Bureau).
- ☒ b. has been transmitted by the International Bureau.
- ☐ c. is not required, as the application was filed in the United States Receiving Office (RO/US).

☐ 6. A **translation** of the International Application into English (35 U.S.C. 371(c)(2)).

☒ 7. Amendments to the claims of the International Appln. under PCT Article 19 (35 USC 371 (c)(3))

- ☐ a. are transmitted herewith (required only if not transmitted by the International Bureau).
- ☐ b. have been transmitted by the International Bureau.
- ☐ c. have not been made; however, the time limit for making such amendments had NOT expired.
- ☒ d. have not been made and will not be made.

☐ 8. A translation of the amendments to the claims under PCT Article 19 (35 U.S.C. 371(c)(3)).

☐ 9. An **oath** or declaration of the inventor(s) (35 U.S.C. 371(c)(4)).

☐ 10. A translation of the annexes to the Int'l Prelim. Exam. Report under PCT Article 36 (35 U.S.C. 371(c)(5)).

Items 11. to 16. below concern document(s) or information included:

☐ 11. An **Information Disclosure Statement** under 37 C.F.R. 1.97 and 1.98.

☐ 12. An **Assignment** document for recording. A separate cover sheet in compliance with 37 CFR 3.28 and 3.31 is included.

☐ 13. A **First preliminary amendment.**

☐ A Second or subsequent preliminary amendment.

☐ 14. A substitute specification.

☐ 15. A change of power of attorney and/or address letter.

☐ 16. Other items or information:

☐

☐ A copy of the Notification of Missing Requirements under 35 U.S.C. 371.

☐ In the event that a petition for extension of time is required to be submitted herewith, and in the event that a separate petition does not accompany this response, applicant hereby petitions under 37 CFR 1.136(a) for an extension of time of as many months as are required to render this submission timely. Any fee is authorized in 17(c).

Date: 05 October 2000

LARSON & TAYLOR, PLC • 1199 North Fairfax St. • Suite 900 • Alexandria, VA 22314

U.S. APPLICATION NO. (if known) 09/647780	INTERNATIONAL APPLICATION NO. PCT/FR99/00807	ATTORNEY DOCKET NO. P06910US00/BAS

	CALCULATIONS *PTO USE ONLY*
☒ 17. **The following fees are submitted:**	
☒ **Basic National Fee** (37 CFR 1.492 (a) (1)-(5):	
☐ Neither Int'l Prelim. Exam. fee nor Int'l Search fee paid to USPTO $1000	
☒ Search Report has been prepared by the EPO or JPO $ 860	
☐ No Int'l Prelim. Ex. fee paid to USPTO but Int'l Search fee paid to USPTO $ 710	
☐ International preliminary examination fee paid to USPTPO $ 690	
☐ Int'l Prelim. Ex. fee paid to USPTO & all claims satisfied PCT Art. 33(1)-(4) $ 100	
ENTER APPROPRIATE BASIC FEE AMOUNT =	$ 860.00
☐ Surcharge of **$130** for furnishing the oath or declaration later than ☐ 20 mos. ☐ 30 mos. + from the earliest claimed priority date (37 CFR 1.492(e)).	$

CLAIMS	*NUMBER FILED*	*NUMBER EXTRA*	*RATE*	
Total Claims	- 20 =		X **$18** =	$
Independent Claims	- 03 =		X **$80** =	$
☐ Multiple Dependent Claim(s) (if applicable)			+ **$270** =	$

TOTAL OF ABOVE CALCULATIONS =	$ 860.00
☐ Reduction of ½ for **small** entity status of applicant.	$
SUBTOTAL =	$ 860.00
☐ Processing fee of **$130** for furnishing the **English translation** later than ☐ 20 mos. ☐ 30 mos. + from the earliest claimed priority date (37 CFR 1.492(f)).	$
TOTAL NATIONAL FEE =	$ 860.00
☐ Fee for recording the enclosed **assignment**, accompanied by a cover sheet - **$40** per property	$
TOTAL FEES ENCLOSED =	$ 860.00
Amount to be *Refunded*	*$*
Amount to be *Charged*	*$*

☒ a. A **check** in the amount of $ 860.00 to cover the above fees is enclosed.

☐ b. Please charge my Deposit Account No. 12-0555 in the amount of $ to cover the above fees.

☒ c. The Commissioner is hereby authorized to charge any additional fees required or credit overpayment to Deposit Account No. 12-0555.

Note: *Where an appropriate time limit under 37 CFR 1.494 or 1.495 has not been met, a petition to revive (37 CFR 1.137(a) or (b)) must be filed and granted to restore the application to pending status.*

SEND ALL CORRESPONDENCE TO:

B. Aaron Schulman

At the address (below) of **CUSTOMER NO. 000881.**

LARSON & TAYLOR, PLC
1199 NORTH FAIRFAX ST.
SUITE 900
ALEXANDRIA, VA 22314

SIGNATURE: Douglas E. Jackson

NAME: DOUGLAS E. JACKSON

REG. NO.: 28518

PHONE NO.: 703-739-4900

Date: 05 OCTOBER 2000

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of:

OUIMET et al.

Serial No.: 09/647,780 — Examiner:

Filed: October 5, 2000 — Art Unit:

National Stage of PCT/FR99/0807

For: NOVEL NEP II MEMBRANE METALOPROTEASE AND ITS USE FOR SCREENING . . . — Docket No.: P06910US0/BAS

STATEMENT UNDER 37 C.F.R. § 1.821

Honorable Assistant Commissioner of Patents and Trademarks

Washington, D.C. 20231

SIR:

I hereby certify in accordance with 37 C.F.R. 1.821(f) that the content of the enclosed paper sequence listing and computer readable form of the sequence listing are the same. In accordance with 37 C.F.R. 1.821(g), I hereby certify that the enclosed submission contains no new matter.

Respectfully submitted,

Date: December 26, 2000

B. Aaron Schulman
Registration No. 31,877

LARSON & TAYLOR, PLC
Transpotomac Plaza
1199 North Fairfax Street
Suite 900
Alexandria, Virginia 22314
(703) 739-4900

#11 CBurt
09/647780

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of:

OUIMET et al.

Serial No.: 09/647,780 — Examiner:

Filed: October 5, 2000 — Art Unit:

National Stage of PCT/FR99/0807

For: NOVEL NEP II MEMBRANE METALOPROTEASE AND ITS USE FOR SCREENING . . . — Docket No.: P06910US0/BAS

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

S I R:

In response to the Notice dated December 4, 2001, please amend the application as follows:

IN THE SPECIFICATION:

After Page 14, please substitute the attached Sequence Listing for any Sequence Listing previously filed in the application.

REMARKS

By this Preliminary Amendment, Applicants are submitting a revised Sequence Listing which overcomes the objections pointed out in the Notice dated December 4, 2001, as well as a copy of the paper sequence in computer readable form.

STATEMENT UNDER 37 CFR § 1.821

Applicants hereby certify in accordance with 37 C.F.R. 1.821(f) that the content of the enclosed paper sequence listing and computer readable form of the sequence listing are the same. In accordance with 37 C.F.R. 1.821(g), Applicants hereby certify that the enclosed submission contains no new matter.

In light of the foregoing, it is submitted that all prior objections have been overcome, and that the present application should be examined and passed on to allowance at the earliest possible time.

Respectfully submitted,

LARSON & TAYLOR, PLC

Date: December 13, 2001

B. Aaron Schulman
Registration No. 31,877

Transpotomac Plaza
1199 N. Fairfax Street
Suite 900
Alexandria, VA 22314
(703) 739-4900

PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re patent application of:

OUIMET et al.

Serial No.: 09/647,780

Filed: October 5, 2000

National Stage of PCT/FR99/0807

For: NOVEL NEP II MEMBRANE METALOPROTEASE AND ITS USE FOR SCREENING . . .

Examiner:

Art Unit:

Docket No.: P06910US0/BAS

PRELIMINARY AMENDMENT

Assistant Commissioner for Patents
Washington, DC 20231

S I R:

Prior to the examination of the above-identified application, please amend the application as follows:

IN THE SPECIFICATION:

After Page 14, please insert the attached Sequence Listing (12 pages) and delete the Sequence Listing previously included in the application.

IN THE CLAIMS:

Please amend Claim 9 as follows:

--9. (Amended) A method for detecting the expression of the NEP II polypeptide in a cell or tissue sample or in cells or a tissue, by *in situ* hybridization, comprising the steps consisting in:

- preparing the RNA of said sample or of said cells or of said tissue;

- bringing said RNA obtained into contact with at least one probe having a nucleotide sequence which is capable of hybridizing specifically with a nucleotide sequence as claimed in claim 2[, said probe possibly being in particular an oligonucleotide probe as claimed in claim 3]; and

- detecting the presence of mRNA hybridizing with said probe, which indicates the expression of the NEP II polypeptide.--

Please amend Claim 12 as follows:

--12. (Amended) A method for detecting NEP II in a cell or tissue sample or in cells or a tissue, comprising the steps consisting in:

- bringing said cell or tissue sample, said cells or said tissue into contact with a compound which is a substrate for the NEP II polypeptide, obtained according to the method of claim 9, or with a compound which is a inhibitor of the metalloprotease activity of NEP II, [obtained according to the screening method of claim 11,] said substrate compound or said inhibitor compound being labeled; and

- detecting the presence of said substrate compound or of said inhibitor compound, which is an indication of the presence of the NEP II polypeptide.--

Please amend Claim 13 as follows:

--13. (Amended) [The use of] A method of using the NEP II polypeptide as claimed in claim 1 for screening compounds which are inhibitors of the metalloprotease activity of NEP II, and which are useful for manufacturing a medicinal product intended for treating disorders involving the peptide transmissions in which NEP II participates.

comprising bringing compounds suspected of being capable of inhibiting the metalloprotease activity of the NEP II polypeptide as claimed in claim 1 into contact with said polypeptide and determining which of said compounds inhibit the metalloprotease activity of said NEP II polypeptide.--

Please amend Claim 14 as follows:

--14. (Amended) [The use] A method of using as claimed in claim 13, [in which] wherein said disorders are [chosen from] selected from the group consisting of cardiovascular and neurodegenerative diseases, growth disorders of endocrine origin, disturbances of the hypothlamo-hypophysial axis and endocrine conditions.--

REMARKS

By this Preliminary Amendment, Applicants are amending Claims 13 and 14 to be more proper under U.S. form and to eliminate multiple dependent claims. In addition, Applicants are providing herewith a computer diskette of the sequence listing and are incorporating into the present specification a paper copy of the sequence listing in computer readable form.

Examination and allowance of the present claims are thus earnestly solicited.

Respectfully submitted,
LARSON & TAYLOR, PLC

Date: December 26, 2000

B. Aaron Schulman
Registration No. 31,877

1199 N. Fairfax Street, Suite 900
Alexandria, VA 22314
(703) 739-4900

"Novel membrane-bound metalloprotease NEP II and the use thereof for screening inhibitors useful in therapy"

The subject of the present invention is a novel membrane-bound metalloprotease called NEP II and the use thereof, in particular for screening inhibitors useful in therapy.

Membrane-bound metalloproteases such as neprilysin (NEP I, EC 3.4.24.11) play an important role in the activation or inactivation of neuronal or hormonal peptide messengers. Their selective inhibition by synthetic compounds has already led to medicinal products which are commonly used in therapeutics, or which are in the process of clinical development, in particular in the gastroenterological (Baumer et al., Gut, 1992, 33: 753-758) and cardiovascular (Gros et al., Proc. Natl. Acad. Sci. USA, 1991, 88: 4210-4214) fields. The isolation of the cDNAs of genes of novel related metalloproteases is likely to enable the development of novel classes of specific inhibitors with promising therapeutic uses. It is in this way that the cloning and the expression of the endothelin-converting enzyme (ECE) gene (Xu et al., Cell, 1994, 78: 473-485) allowed the development of inhibitors which are potentially useful in certain cardiovascular disorders.

The authors of the present invention have revealed a novel membrane-bound metalloprotease belonging to the ECE/NEP/Kell family (Lee S. et al., 1991, PNAS 88(14): 6353-57), which they have called NEP II.

A subject of the present invention is thus an isolated polypeptide comprising an amino acid sequence chosen from the sequence SEQ ID No. 2 or SEQ ID No. 4, a sequence derived from or homologous to said sequence SEQ ID No. 2 or SEQ ID No. 4, and a biologically active fragment of said sequence SEQ ID No. 2 or SEQ ID No. 4,

said isolated polypeptide being referred to as "NEP II".

The sequence SEQ ID No. 2 is the amino acid sequence of NEP II identified in rats.

The sequence SEQ ID No. 4 is an amino acid sequence (partial) of NEP II identified in humans.

The term "derived" polypeptide is intended to mean any polypeptide resulting from a modification of genetic and/or chemical type of the sequence SEQ ID No. 2 or SEQ ID No. 4, i.e. by mutation, deletion, addition, substitution and/or chemical modification of at least one amino acid, or any isoform having a sequence identical to the sequence SEQ ID No. 2 or SEQ ID No. 4, but containing at least one amino acid in the D form.

Said substitutions are preferably conservative substitutions, i.e. substitutions of amino acids of the same class, such as substitutions of amino acids with uncharged side chains (such as asparagine, glutamine, serine, threonine or tyrosine), of amino acids with basic side chains (such as lysine, arginine or histidine), of amino acids with acidic side chains (such as aspartic acid or glutamic acid) or of amino acids with apolar side chains (such as glycine, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan or cysteine).

The term "homologous" polypeptide is intended to mean more particularly any polypeptide which can be isolated from mammalian species other than rats or humans.

Said homologous polypeptides show preferably greater than 70%, even more preferably greater than 75%, sequence homology with the complete sequence SEQ ID No. 2 or SEQ ID No. 4, the homology being particularly high in that portion of said polypeptide containing the active site.

The homology is generally determined using a sequence analysis software package (for example, Sequence Analysis Software Package of the Genetics

Computer Group, University of Wisconsin Biotechnology Center, 1710 University Avenue, Madison, WI 53705). Similar amino acid sequences are aligned in order to obtain the maximum degree of homology (i.e. identity). To this end, it may be necessary to artificially introduce "gaps" into the sequence. Once the optimum alignment has been produced, the degree of homology (i.e. identity) is established by recording all the positions for which the amino acids of the two compared sequences are identical, with respect to the total number of positions.

Said polypeptides derived from or homologous to, or the polypeptide fragments of, the polypeptide of sequence SEQ ID No. 2 or SEQ ID No. 4 are biologically active, i.e. they have biological properties identical or similar of the biological properties of the NEP II polypeptide of sequence SEQ ID No. 2 or SEQ ID No. 4, namely metalloprotease activity.

The preferred polypeptide fragments comprise the sequence of the active site responsible for binding the zinc atom which is essential for the catalysis. This active site has been identified as encompassing the HEX_1X_2H, X_1 and X_2 residues representing varied amino acids. It is in particular the HEITH sequence (amino acids 608 to 612 of the sequence SEQ ID No. 2) in the NEP II polypeptide in rats and humans.

A subject of the present invention is also an isolated nucleic acid comprising a nucleotide sequence chosen from the sequence SEQ ID No. 1 or SEQ ID No. 3, a sequence derived from or homologous to said sequence SEQ ID No. 1 or SEQ ID No. 3, and the complementary sequences thereof.

The sequence SEQ ID No. 1 is the cDNA sequence comprising the coding frame for NEP II identified in rats.

The sequence SEQ ID No. 3 is the cDNA sequence comprising (partially) the coding frame for NEP II identified in humans.

The term "derived" nucleotide sequence is intended to mean any nucleotide sequence encoding a polypeptide derived from NEP II as defined above, i.e. a sequence resulting from a modification of the sequence SEQ ID No. 1 or SEQ ID No. 3, in particular by mutation, deletion, addition or substitution of at least one nucleotide. Included in particular are the sequences which are derived from the sequence SEQ ID No. 1 or SEQ ID No. 3 by degeneracy of the genetic code.

The term "homologous" sequence is intended to mean more particularly any nucleotide sequence encoding an NEP II polypeptide homologous to the NEP II polypeptide of sequence SEQ ID No. 2 or SEQ ID No. 4 in mammalian species other than rats or humans.

Such a homologous sequence has preferably greater than 70%, even more preferably greater than 75%, homology with the sequence SEQ ID No. 1 or SEQ ID No. 3, the homology being particularly high in the central portion of the sequence encoding the NEP II polypeptide.

Preferably, such as homologous nucleotide sequence hybridizes specifically with the sequences which are complementary to the sequence SEQ ID No. 1 or No. 3, under stringent conditions. The parameters which define the stringency conditions depend on the temperature at which 50% of the paired strands separate (Tm).

For sequences comprising more than 30 bases, Tm is defined by the equation: $Tm=81.5+0.41(\%G+C)+16.6\mathrm{Log}(\text{concentration of cations}) - 0.63(\%\text{formamide}) - (600/\text{number of bases})$ (Sambrook et al., Molecular Cloning, A laboratory manual, Cold Spring Harbor laboratory Press, 1989, pages 9.54-9.62).

For sequences more than 30 bases long, Tm is defined by the equation: $Tm=4(G+C) + 2(A+T)$.

Under suitable stringency conditions, under which the nonspecific sequences do not hybridize, the hybridization temperature is approximately 5 to 30°C,

preferably 5 to 15°C, below Tm, even more preferably 5 to 10°C below Tm (high stringency), and the hybridization buffers used are preferably solutions with high ionic strength, such as a 6xSSC solution for example.

The nucleotide sequences according to the invention can be used for producing a recombinant NEP II protein according to the invention, according to techniques for producing recombinant products, known to persons skilled in the art.

An effective system for producing a recombinant protein must have a vector, for example of plasmid or viral origin, and a compatible host cell.

The cellular host can be chosen from prokaryotic systems such as bacteria, or eukaryotic systems such as, for example, yeasts, insect cells or mammalian cells, for instance CHO cells (Chinese hamster ovary cells), or any other advantageously available system.

The vector should comprise a promoter, translation initiation and termination signals, and the suitable transcription regulation regions. It should be able to be integrated into the cell and can optionally have specific signals determining the secretion of the translated protein.

These various control signals are chosen according to the cellular host used. For this purpose, the nucleotide sequences according to the invention can be inserted into vectors which replicate autonomously within the chosen host, or vectors which integrate in the chosen host. Such vectors will be prepared according to the methods commonly used by persons skilled in the art, and the clones resulting therefrom can be introduced into a suitable host by standard methods, such as for example electroporation.

Examples of vectors of interest are the plasmids pcDNA 3.1, PCR2.1 (Invitrogen), or pMbac (Stratagene).

The invention is aimed toward the cloning and/or expression vectors containing a nucleotide sequence according to the invention, and is also aimed toward the host cells transfected with these vectors. These cells can be obtained by introducing into host cells a nucleotide sequence inserted into a vector as defined above, and then culturing said cells under conditions which allow the replication and/or expression of the transfected nucleotide sequence.

These cells can be used in a method for producing a recombinant polypeptide according to the invention.

The method for producing a polypeptide of the invention in recombinant form is itself included in the present invention, and is characterized in that the transfected cells are cultured under conditions which allow the expression of a recombinant polypeptide according to the invention, and in that said recombinant polypeptide is recovered.

The purification methods used are known to persons skilled in the art. The recombinant polypeptide can be purified from cell lysates and extracts, or from the culture medium supernatant, by methods used separately or in combination, such as fractionation, chromatography methods, or immunoaffinity techniques using monoclonal antibodies or polyclonal serum, etc.

A subject of the present invention is also the nucleotide probes which are capable of hybridizing strongly and specifically with a nucleic acid sequence, of a genomic DNA or of a messenger RNA, encoding a polypeptide according to the invention. The suitable hybridization conditions correspond to the temperature and ionic strength conditions conventionally used by persons skilled in the art (Sambrook et al., 1989), preferably to conditions of high stringency, i.e. temperature conditions between (Tm minus 5°C) and (Tm minus 15°C) and even more preferably to temperature conditions between Tm and (Tm minus 10°C) (high stringency).

The preferred probes are in particular the oligonucleotide probes chosen from the sequences SEQ ID No. 5 to SEQ ID No. 27.

Such probes are useful for sequencing reactions or specific amplification reactions according to the so-called PCR (polymerase chain reaction) technique or any other variant of this.

Such probes are also useful in a method for detecting the expression of the NEP II polypeptide in a cell or tissue sample or in cells or a tissue, by *in situ* hybridization, comprising the steps consisting in:

- preparing the RNA of said sample or of said cells or of said tissue;
- bringing said RNA obtained into contact with at least one probe having a nucleotide sequence which is capable of hybridizing specifically with a nucleotide sequence according to the invention, said probe possibly being in particular an oligonucleotide probe of sequence SEQ ID No. 5 to SEQ ID No. 27;
- detecting the presence of mRNA hybridizing with said probe, which indicates the expression of the NEP II polypeptide.

A subject of the invention is also mono- or polyclonal antibodies or their fragments, chimeric antibodies or immunoconjugates, characterized in that they are obtained using a polypeptide according to the invention administered to an animal, and are capable of recognizing specifically a polypeptide according to the invention. A subject of the invention is also the use of these antibodies for purifying or detecting an NEP II polypeptide in a biological sample.

The polyclonal antibodies can be obtained from the serum of an animal immunized against the NEP II protein produced, for example, by genetic recombination using the method described above, according to the usual procedures.

The monoclonal antibodies can be obtained according to the conventional method of hybridoma

culturing described by Köhler and Milstein (Nature, 1975, vol. 256, pp 495-497).

The antibodies can be chimeric antibodies, humanized antibodies or Fab and F(ab')2 fragments. They can also be in the form of labeled antibodies or immunoconjugates.

The antibodies according to the invention are particularly useful for detecting the presence of NEP II.

A subject of the present invention is therefore a method for immunologically detecting NEP II in a cell or tissue sample or in cells or a tissue, comprising the steps consisting in:

- bringing said cell or tissue sample, said cells or said tissue into contact with a detectable antibody according to the invention;
- detecting the presence of said antibody, which is an indication of the presence of the NEP II polypeptide.

The term "detectable antibody" is intended to mean either an antibody labeled with a detectable group, such as a group which is radioactive, enzymatic, fluorogenic or fluorescent, or an antibody to which another antibody, which is itself labeled in a detectable manner is bound.

The antibodies according to the invention can thus make it possible to evaluate overexpression of the ~~[lacuna]~~ NEP II polypeptide, which may be an indication of neuroendocrine tumour cells in particular.

A subject of the invention is also a method for identifying compounds which are substrates for the NEP II polypeptide as defined above, in which said compounds, optionally labeled, are brought into contact with the NEP II polypeptide, and the cleavage of said compounds by NEP II, which is an indication of the metalloprotease activity of NEP II toward said substrate compounds, is evaluated.

Such substrates specific for NEP II can in particular be used in a method for detecting the

metalloprotease activity of NEP II in a cell or tissue sample or in cells or a tissue, comprising the steps consisting in:

- bringing said cell or tissue sample, said cells or said tissue into contact with a compound which is a substrate for the NEP II polypeptide, obtained according to the invention, said substrate compound being optionally labeled;

- evaluating the cleavage of said substrate compound, which is an indication of the metalloprotease activity of NEP II.

Cells which can be thus assayed are especially cells transfected with a polynucleotide encoding the NEP II polypeptide as defined above. Tissue extracts which can be assayed are especially testicle membranes, which are particularly rich in NEP II metalloprotease.

A subject of the invention is, moreover, a method for screening compounds which are capable of inhibiting the metalloprotease activity of the NEP II polypeptide according to the invention, in which said compounds are brought into contact with said NEP II polypeptide and the degree of inhibition of the metalloprotease activity of NEP II is evaluated.

The compounds capable of inhibiting the metalloprotease activity of NEP II are preferably short peptides of 2 or 3 natural or modified amino acids.

The synthetic peptides identified as inhibitors of the metalloprotease activity of NEP II by this screening method can be coupled to a zinc-chelating group, such as thiol, phosphate or hydroxamic acid groups, according to the conventional techniques known to persons skilled in the art. The inhibitor compound obtained is a good candidate as an active principle of a medicinal product, in combination with a pharmaceutically acceptable vehicle. Said chelating group can optionally be transiently protected, for example with a thiol ester, so as to improve the bioavailability of said active principle.

The NEP II polypeptide according to the invention is particularly useful for screening compounds which are inhibitors of the metalloprotease activity of NEP II and which are useful for manufacturing a medicinal product intended for treating disorders involving peptide transmissions in which NEP II participates.

Among the disorders under consideration, mention may be made in particular of cardiovascular and neurodegenerative diseases, growth disorders of endocrine origin, disturbances of the hypothalamo-hypophysial axis and endocrine conditions. More particularly targeted are disorders affecting the metabolism of neurohormones or factors of the corticotropic sphere.

The compounds which are substrates for NEP II or which are inhibitors of the metalloprotease activity of NEP II, obtained according to the methods described above, can also be useful for detecting the NEP II protein.

A subject of the present invention is therefore also a method for detecting NEP II in a cell or tissue sample or in cells or a tissue, comprising the steps consisting in:

- bringing said cell or tissue sample, said cells or said tissue into contact with a compound which is a substrate for the NEP II polypeptide, obtained as defined above, or with a compound which is an inhibitor of the metalloprotease activity of NEP II, obtained according to the screening method as defined above, said substrate compound or said inhibitor compound being labeled;

- detecting the presence of said substrate compound or of said inhibitor compound, which is an indication of the presence of the NEP II polypeptide.

The term "labeled substrate compound" or "labeled inhibitor" is intended to mean a substrate compound or an inhibitor compound which is labeled in a detectable manner, for example with a group which is

radioactive, enzymatic, fluorogenic or fluorescent, etc.

The following examples illustrate the invention without limiting it.

EXAMPLE 1:

Cloning the cDNA encoding NEP II in rats

Degenerate oligonucleotides were obtained based on the alignment of the peptide sequences of the ECE, NEP I and Kell enzymes, and on the delimitation of the regions of strong homology.

The total RNA of various rat tissues (brain, intestine and testicles) was subjected to reverse transcription (RT) and amplified by polymerase chain reaction (PCR), using a pair of degenerate oligonucleotides, over the N-terminal region rich in cysteine residues:

The sequences of these degenerate oligonucleotides are as follows:

DCYS2 CCC AAG (G/T)CG (A/G)G(A/G) CTG GTC

DCYS3 T(A/T)(C/T) GC(A/C/T/G) GG(A/T) GG(A/C) TGG

This made it possible to amplify a 420-base pair fragment from the testicle RNAt, encoding an open reading frame which has 76% homology with the NEP I protein. This sequence was completed by 3′ and 5′ RACE (rapid amplification of cDNA ends), using RNAt from brain and from testicles. The sequences were confirmed by verifying five different clones for each tissue and each amplification. The complete cDNA (SEQ ID No. 1) was then cloned into the vectors PCR2.1 and pcDNA3.1 (Invitrogen).

EXAMPLE 2:

Characteristics of the rat NEP II polypeptide

The novel gene isolated encodes a 774-amino acid protein (SEQ ID No. 2) which, besides strong homologies with the NEP I, ECE and Kell enzymes (52%, 40% and 28% amino acid identity, respectively), has the consensus sequence of the HEXXH active site, a

transmembrane region (amino acids 24 to 40 in the sequence SEQ ID No. 2) followed by four cysteine residues which are characteristic of this family, and seven potential glycosylation sites. Three alternative splicings were identified by sequencing the RACE products and by RT-PCR. One of these alternative splicings eliminates a potential glycosylation site and might affect the transit of the protein to the surface of the cell, or its activity. Each splicing corresponds, moreover, to an exon of NEP I, which suggests a similar gene structure. These data demonstrate that this novel enzyme belongs to the family of ECE/NEP/Kell metalloproteases. Its notable homology with NEP I led to it being named NEP II.

EXAMPLE 3:

Cloning the cDNA encoding NEP II in humans

In order to clone the human homologue of NEP II, two oligonucleotides were designed, based on the protein sequence of rat NEP II. The sequences were chosen, on the one hand, for their low degeneracy (such as, for example, a tryptophan, represented by a single codon in the genetic code) and, on the other hand, for their degree of conservation (such as the zinc binding site).

1- (H)EITHFD (SEQ IDNo.28) or 5' - CGA GAT CAC ACA TGG CTT TGA TGA – 3' (S) (SEQ IDNo.22)

2- QVWCGS (SEQ IDNo.29) or 5'- GGA CCC ACA CCA CAC CTG – 3' (AS) (SEQ ID n°23)

A polymerase chain reaction was carried out on human hippocampe cDNA obtained from a library (Stratagene), and a 330-bp band was amplified, subcloned and sequenced (SEQ ID No. 3). The sequence obtained shows 82% sequence homology with rat NEP II, which makes it possible to assert that it encodes the human homologue.

The presence of the HEITH zinc binding site was confirmed by 5′ RACE using the human-specific HNII-2 and HNII-3 oligonucleotides. Similarly, the HNII-1 and HNII-2 oligonucleotides will enable the amplification of the 3′ region by the 3′ RACE technique.

HNII-1 5'- CGG CCT GGA TCT CAC CCA TGA G – 3' (SEQ IDNo.24)

HNII-2 5'- CTG ACT GCT CCC GGA AGT GCT GGG TG – 3' (SEQ IDNo.25)

HNII-3 5'- GAG CAG CTC TTC TTC ATC – 3' (SEQ IDNo.26)

HNII-4 5'- CTC CAC CAA TCC ATC ATG TTG C – 3' (SEQ IDNo.27).

EXAMPLE 4:

NEP II tissue expression

Northern blot and RT-PCR studies show that NEP II is encoded by a 2.8-Kb transcript which is very highly expressed in rat testicles, and moderately expressed in the heart, the liver, the digestive sytem and the brain. Semi quantitative RT-PCR studies show a similar expression profile in these tissues and a predominance of the long forms.

All these characteristics indicate clearly that the protein identified for the first time is a membrane-bound metalloprotease (ectoprotease) responsible for the metabolism of neuronal and/or hormonal messenger peptides.

The native NEP II polypeptide is expressed in a heterogeneous manner in the nervous system, the glands (hypophyses, testicle), the digestive apparatus (small intestine in particular) and the cardiovascular system (heart in particular).

In situ hybridization techniques also indicate a high expression of the NEP II protein in neurons and adenohypophysial cells expressing the gene for POMC (propiomelanocortin), which is the precursor of ACTH.

These locations indicate the participation of NEP II in the proteolysis of hormones and of peptide neurotransmitters, or of their precursors, coming from or acting on these diverse organs. It consequently becomes advantageous, for therapeutic purposes, to affect the corresponding peptide transmissions by inhibiting NEP II.

CLAIMS

1. An isolated polypeptide comprising an amino acid sequence chosen from the sequence SEQ ID No. 2 or SEQ ID No. 4, a sequence derived from or homologous to said sequence SEQ ID No. 2 or SEQ ID No. 4, and a biologically active fragment of said sequence SEQ ID No. 2 or SEQ ID No. 4, said isolated polypeptide being referred to as "NEP II".

2. An isolated nucleic acid comprising a nucleotide sequence chosen from the sequence SEQ ID No. 1 or SEQ ID No. 3, a sequence derived from or homologous to said sequence SEQ ID No. 1 or SEQ ID No. 3, and the complementary sequences thereof.

3. An oligonucleotide probe which hybridizes specifically with a nucleotide sequence as claimed in claim 2, said probe having a nucleotide sequence chosen from the sequences SEQ ID No. 5 to SEQ ID No. 27.

4. A cloning and/or expression vector containing a nucleotide sequence as claimed in claim 2.

5. A host cell transfected with a vector as claimed in claim 4.

6. Mono- or polyclonal antibodies or their fragments, chimeric antibodies or immunoconjugates, characterized in that they are obtained using a polypeptide as claimed in claim 1 administered to an animal, and are capable of recognizing specifically a polypeptide as claimed in claim 1.

7. A method for immunologically detecting NEP II in a cell or tissue sample or in cells or a tissue, comprising the steps consisting in:

- bringing said cell or tissue sample, said cells or said tissue into contact with a detectable antibody as claimed in claim 6;
- detecting the presence of said antibody, which is an indication of the presence of the NEP II polypeptide.

8. A method for detecting the expression of the NEP II polypeptide in a cell or tissue sample or in cells or a tissue, by *in situ* hybridization, comprising the steps consisting in:

- preparing the RNA of said sample or of said cells or of said tissue;

- bringing said RNA obtained into contact with at least one probe having a nucleotide sequence which is capable of hybridizing specifically with a nucleotide sequence as claimed in claim 2, said probe possibly being in particular an oligonucleotide probe as claimed in claim 3;

- detecting the presence of mRNA hybridizing with said probe, which indicates the expression of the NEP II polypeptide.

9. A method for identifying compounds which are substrates for the NEP II polypeptide as claimed in claim 1, in which said compounds, optionally labeled, are brought into contact with the NEP II polypeptide, and the cleavage of said compounds by NEP II, which is an indication of the metalloprotease activity of NEP II toward said substrate compounds, is evaluated.

10. A method for detecting the metalloprotease activity of NEP II in a cell or tissue sample or in cells or a tissue, comprising the steps consisting in:

- bringing said cell or tissue sample, said cells or said tissue into contact with a compound which is a substrate for the NEP II polypeptide, obtained according to the method of claim 9, said substrate compound being optionally labeled;

- evaluating the cleavage of said substrate compound, which is an indication of the metalloprotease activity of NEP II.

11. A method for screening compounds which are capable of inhibiting the metalloprotease activity of the NEP II polypeptide as claimed in claim 1, in which said compounds are brought into contact with said NEP II polypeptide and the degree of inhibition of the metalloprotease activity of NEP II is evaluated.

12. A method for detecting NEP II in a cell or tissue sample or in cells or a tissue, comprising the steps consisting in:

- bringing said cell or tissue sample, said cells or said tissue into contact with a compound which is a substrate for the NEP II polypeptide, obtained according to the method of claim 9, or with a compound which is an inhibitor of the metalloprotease activity of NEP II, obtained according to the screening method of claim 11, said substrate compound or said inhibitor compound being labeled;

- detecting the presence of said substrate compound or of said inhibitor compound, which is an indication of the presence of the NEP II polypeptide.

13. The use of the NEP II polypeptide as claimed in claim 1 for screening compounds which are inhibitors of the metalloprotease activity of NEP II, and which are useful for manufacturing a medicinal product intended for treating disorders involving the peptide transmissions in which NEP II participates.

14. The use as claimed in claim 13, in which said disorders are chosen from cardiovascular and neurodegenerative diseases, growth disorders of endocrine origin, disturbances of the hypothalamo-hypophysial axis and endocrine conditions.

ATTORNEY/DOCKET NO: Fuertm 9801329

DECLARATION FOR PATENT APPLICATION AND APPOINTMENT OF ATTORNEY

As a below named inventor, I hereby declare that my residence, post office address and citizenship are as stated below next to my name; I believe that I am the original, first and sole inventor (if only one name is listed below) or an original, first and joint inventor (if plural names are listed below) of the subject matter which is claimed and for which a patent is sought on the invention (Design, if applicable) entitled "Novel membrane-bound metalloprotease NEP II and the use thereof for screening inhibitors useful in therapy".

the specification of which (check one):

☐ is attached hereto.

☒ was filed on October 5, 2000 as Application Serial No 09/647,780, and was amended on _____ (if applicable).

☒ was filed on April 7, 1999 as International Application (PCT) No. PCT/FR99/00807, and was amended on _____ (if applicable).

I hereby state that I have reviewed and understand the contents of the above-identified specification, including the claims, as amended by any amendment(s) referred to above. I acknowledge the duty to disclose information which is material to the examination of this application in accordance with *Title 37, Code of Federal Regulations, §1.56(a)*. I hereby claim foreign priority benefits under *Title 35, United States Code §119* of any foreign application(s) for patent or inventor's certificate listed below and have also identified below any foreign application for patent or inventor's certificate having a filing date before that of the application on which priority is claimed.

PRIOR FOREIGN APPLICATION(S)			*PRIORITY CLAIMED*	
98 04 389 (Number)	FRANCE (Country)	April 8, 1998 (Day/Month/Year Filed)	☒ Yes	☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes	☐ No
(Number)	(Country)	(Day/Month/Year Filed)	☐ Yes	☐ No

I hereby claim the benefit under *Title 35, United States Code, §120* of any United States application(s) or PCT international application(s) designating The United States of America listed below and, insofar as the subject matter of each of the claims of this application is not disclosed in that/those prior application(s) in the manner provided by the first paragraph of *Title 35, United States Code, §112*, I acknowledge the duty to disclose material information as defined in *Title 37, Code of Federal Regulations, §1.56(a)* which occurred between the filing date of the prior application(s) and the national or PCT international filing date of this application:

Application Number	Filing Date	Status - Patented, Pending or Abandoned

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under *section 1001 of title 18 of the United States Code* and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

POWER OF ATTORNEY: I (We) hereby appoint as my (our) attorneys, with full powers of substitution and revocation, to prosecute this application and transact all business in the Patent and Trademark Office connected therewith: Andrew E.Taylor, R 7000, Thomas P.Sarro 19396-Harold L.Novick 26011-Mark J.Guttag 33057, Walter C.Gillis 22086, Ross Hunt Jr 25082, Douglas E.Jackson 28518, Daniel C.Mallery 33532, Marvin Petry 22752, William E.Jac -016, B.Aaron Schulman 31877

Telephone Calls to: 703 920 7200.

Send correspondence to: LARSON and TAYLOR - Transpotomac Plaza - 1199 North Fairfax street, suite 900 - Alexandr VIRGINIA 22314 - USA

☐ *See following pages for additional joint inventors.*

Full Name of First or Sole Inventor	*Citizenship*
OUIMET Tanja	CANADIAN
Residence Address Street	*Post Office Address Street*
3 rue Jules César	
City	*City*
PARIS	
State or Country / *Zip*	*State or Country* / *Zip*
FRANCE TRX / 75012	
DATE	*SIGNATURE*
30 10 00	Tanja Ouimet

BACON & THOMAS
EWR0003 - PATAPATT.DEC

Full Name of Joint Inventor: Gros Claude	Citizenship: French
Residence Address Street: 31 rue de Flore	Post Office Address Street:
City: PARIS	City:
State or Country: FRANCE Zip: FRX 75015	State or Country: Zip:
DATE: October 30, 2000	SIGNATURE:

Full Name of Joint Inventor: ROSE Christiane	Citizenship: French
Residence Address Street: 20 place Henri IV	Post Office Address Street:
City: Le Nesnil St Denis	City:
State or Country: FRANCE Zip: FRX 78320	State or Country: Zip:
DATE: October 30, 2000	SIGNATURE:

Full Name of Joint Inventor: BONHOMME Marie-Chantal	Citizenship: CANADIAN.
Residence Address Street: 910 Lapointe	Post Office Address Street:
City: St-Laurent,	City:
State or Country: CANADA Zip: P.Q. H4L 1J8	State or Country: Zip:
DATE: October 30, 2000	SIGNATURE:

Full Name of Joint Inventor: FACCHINETTI Patricia	Citizenship: French
Residence Address Street: 316 AV. du Gal de Gaulle	Post Office Address Street:
City: le Plessis Trevise	City:
State or Country: FRANCE Zip: FRX 94420	State or Country: Zip:
DATE: October 30, 2000	SIGNATURE:

Full Name of Joint Inventor: Schwartz Jean-Charles	Citizenship: French
Residence Address Street: 9, Villa Seurat	Post Office Address Street:
City: PARIS	City:
State or Country: France Zip: FRX 75014	State or Country: Zip:
DATE: 30 oct. 2000	SIGNATURE:

☐ See following pages for additional joint inventors

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Gly Gly Trp Pro Val Ala Met Asp Lys Trp Asn Glu Thr Met Gly Pro
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Lys Trp Glu Leu Glu Arg Gln Leu Ala Val Leu Asn Ser Gln Phe Asn
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Arg Arg Val Leu Ile Asp Leu Phe Ile Trp Asn Asp Asp Gln Asn Ser
225                 230                 235                 240
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Ser Arg His Val Ile Tyr Ile Asp Gln Pro Thr Leu Gly Met Pro Ser
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Leu Gln Phe Met Thr Ser Val Ala Thr Met Leu Arg Arg Asp Leu Asn
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Leu Pro Gly Glu Thr Asp Leu Val Gln Glu Glu Met Ala Gln Val Leu
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His Leu Glu Thr His Leu Ala Asn Ala Thr Val Pro Gln Glu Lys Arg
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His Asp Val Thr Ala Leu Tyr His Arg Met Gly Leu Glu Glu Leu Gln
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Glu Arg Phe Gly Leu Lys Gly Phe Asn Trp Thr Leu Phe Ile Gln Asn
            340                 345                 350

Val Leu Ser Ser Val Gln Val Glu Leu Leu Pro Asn Glu Glu Val Val
        355                 360                 365

Val Tyr Gly Ile Pro Tyr Leu Glu Asn Leu Glu Glu Ile Ile Asp Val
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Phe Pro Ala Gln Thr Leu Gln Asn Tyr Leu Val Trp Arg Leu Val Leu
385                 390                 395                 400

Asp Arg Ile Gly Ser Leu Ser Gln Arg Phe Lys Glu Ala Arg Val Asp
                405                 410                 415

Tyr Arg Lys Ala Leu Tyr Gly Thr Thr Met Glu Glu Val Arg Trp Arg
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Glu Cys Val Ser Tyr Val Asn Ser Asn Met Glu Ser Ala Val Gly Ser
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Leu Tyr Ile Lys Arg Ala Phe Ser Lys Asp Ser Lys Ser Ile Val Ser
    450                 455                 460
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Arg Ser Leu Lys Lys Leu Arg Glu Lys Val Asp Gln Asn Leu Trp Ile
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565 570 575

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580 585 590

Gln Pro Gln Ala Leu Asn Phe Gly Gly Ile Gly Met Val Ile Gly His
595 600 605

Glu Ile Thr His Gly Phe Asp Asp Asn Gly Arg Asn Phe Asp Lys Asn
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625 630 635 640

Gln Gln Ser Gln Cys Met Ile Tyr Gln Tyr Ser Asn Phe Ser Trp Glu
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Leu Ala Asp Asn Gln Asn Val Asn Gly Phe Ser Thr Leu Gly Glu Asn
660 665 670

Ile Ala Asp Asn Gly Gly Val Arg Gln Ala Tyr Lys Ala Tyr Leu Gln
675 680 685

Trp Leu Ala Glu Gly Gly Arg Asp Gln Arg Leu Pro Gly Leu Asn Leu
690 695 700

Thr Tyr Ala Gln Leu Phe Phe Ile Asn Tyr Ala Gln Val Trp Cys Gly
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Ser Tyr Arg Pro Glu Phe Ala Ile Gln Ser Ile Lys Thr Asp Val His
725 730 735

Ser Pro Leu Lys Tyr Arg Val Leu Gly Ser Leu Gln Asn Leu Pro Gly
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AACACCCTTG GGGAAAACAT TGCTGACAAC GGAGGGGTGC GGCAAGCCTA TAAGGCCTAC 240

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Phe Arg Glu Gln Ser Glu Cys Met Ile Tyr Gln Tyr Gly Asn Tyr Ser
35 40 45

Trp Asp Leu Ala Asp Glu Gln Asn Val Asn Gly Phe Asn Thr Leu Gly
50 55 60

Glu Asn Ile Ala Asp Asn Gly Gly Val Arg Gln Ala Tyr Lys Ala Tyr
65 70 75 80

Leu Lys Trp Met Ala Glu Gly Gly Lys Asp Gln Gln Leu Pro Gly Leu
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<130> P06910US0/BAS

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                                                    Met Gly Lys
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TCG GAG AGC TCA GTG GGG ATG ATG GAG AGA GCG GAC AAC TGT GGG AGG         163
Ser Glu Ser Ser Val Gly Met Met Glu Arg Ala Asp Asn Cys Gly Arg
      5                  10                  15

AGG CGC CTA GGC TTC GTG GAG TGT GGG CTG CTG GTA CTG CTG ACA CTG         211
Arg Arg Leu Gly Phe Val Glu Cys Gly Leu Leu Val Leu Leu Thr Leu
 20                  25                  30                  35

CTG TTG ATG GGA GCC ATA GTG ACT CTG GGT GTC TTC TAC AGC ATA GGG         259
Leu Leu Met Gly Ala Ile Val Thr Leu Gly Val Phe Tyr Ser Ile Gly
                 40                  45                  50

AAG CAG CTG CCC CTC TTA AAT AGC CTG CTG CAC GTC TCC CGG CAT GAG         307
Lys Gln Leu Pro Leu Leu Asn Ser Leu Leu His Val Ser Arg His Glu
             55                  60                  65

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         70                  75                  80
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ATC TGT ACT ACC CCA AGC TGC GTG ATA GCA GCT GCC AGA ATC CTC CAG        403
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     85                  90                  95

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Asn Met Asp Gln Ser Lys Lys Pro Cys Asp Asn Phe Tyr Gln Tyr Ala
100                 105                 110                 115

TGC GGA GGC TGG CTA CGG CAC CAT GTG ATC CCC GAG ACC AAC TCC AGA        499
Cys Gly Gly Trp Leu Arg His His Val Ile Pro Glu Thr Asn Ser Arg
                120                 125                 130

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Tyr Ser Val Phe Asp Ile Leu Arg Asp Glu Leu Glu Val Ile Leu Lys
            135                 140                 145

GGG GTG CTG GAG GAT TCC TCT GTC CAG CAC CGC CCA GCT GTG GAG AAG        595
Gly Val Leu Glu Asp Ser Ser Val Gln His Arg Pro Ala Val Glu Lys
        150                 155                 160

GCC AAG ACA CTG TAC CGC TCC TGC ATG AAC CAG AGT GTG ATA GAG AAG        643
Ala Lys Thr Leu Tyr Arg Ser Cys Met Asn Gln Ser Val Ile Glu Lys
    165                 170                 175

AGA GAC TCT GAG CCC CTG CTG AAC GTC TTA GAT ATG ATA GGA GGT TGG        691
Arg Asp Ser Glu Pro Leu Leu Asn Val Leu Asp Met Ile Gly Gly Trp
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CCT GTA GCC ATG GAC AAG TGG AAT GAG ACC ATG GGC CCC AAG TGG GAA        739
Pro Val Ala Met Asp Lys Trp Asn Glu Thr Met Gly Pro Lys Trp Glu
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CTC ATC GAC CTC TTC ATC TGG AAT GAT GAC CAG AAC TCC AGC CGG CAC        835
Leu Ile Asp Leu Phe Ile Trp Asn Asp Asp Gln Asn Ser Ser Arg His
        230                 235                 240

GTC ATC TAC ATA GAC CAG CCC ACC TTG GGC ATG CCC TCC CGG GAG TAC        883
Val Ile Tyr Ile Asp Gln Pro Thr Leu Gly Met Pro Ser Arg Glu Tyr
    245                 250                 255

TAT TTC AAG GAA GAC AGC CAC CGG GTA CGG GAA GCC TAC CTG CAG TTC        931
Tyr Phe Lys Glu Asp Ser His Arg Val Arg Glu Ala Tyr Leu Gln Phe
260                 265                 270                 275

ATG ACA TCA GTG GCC ACT ATG CTG AGG AGA GAC CTG AAC CTG CCC GGG        979
Met Thr Ser Val Ala Thr Met Leu Arg Arg Asp Leu Asn Leu Pro Gly
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GAG ACC GAT TTG GTG CAG GAG GAA ATG GCA CAG GTG CTG CAT CTG GAG       1027
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ACA CAT CTG GCC AAC GCC ACG GTC CCC CAG GAG AAA AGG CAT GAT GTC       1075
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Thr His Leu Ala Asn Ala Thr Val Pro Gln Glu Lys Arg His Asp Val
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Thr Ala Leu Tyr His Arg Met Gly Leu Glu Glu Leu Gln Glu Arg Phe
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GGT CTG AAG GGG TTT AAC TGG ACT CTC TTC ATA CAA AAC GTG CTG TCT      1171
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TCT GTG CAA GTT GAG CTG CTC CCG AAT GAG GAG GTG GTG GTC TAT GGC      1219
Ser Val Gln Val Glu Leu Leu Pro Asn Glu Glu Val Val Val Tyr Gly
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ATC CCC TAC CTG GAG AAT CTT GAG GAG ATC ATT GAC GTC TTC CCA GCA      1267
Ile Pro Tyr Leu Glu Asn Leu Glu Glu Ile Ile Asp Val Phe Pro Ala
            375                 380                 385

CAG ACC TTG CAA AAC TAC CTG GTG TGG CGC CTG GTG CTA GAT CGC ATC      1315
Gln Thr Leu Gln Asn Tyr Leu Val Trp Arg Leu Val Leu Asp Arg Ile
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GGC AGC CTG AGC CAG AGA TTC AAA GAA GCG CGT GTG GAC TAC CGC AAG      1363
Gly Ser Leu Ser Gln Arg Phe Lys Glu Ala Arg Val Asp Tyr Arg Lys
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GCG CTG TAC GGT ACA ACC ATG GAG GAA GTA CGC TGG CGG GAG TGT GTC      1411
Ala Leu Tyr Gly Thr Thr Met Glu Glu Val Arg Trp Arg Glu Cys Val
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AGC TAT GTC AAC AGC AAC ATG GAG AGT GCC GTG GGC TCC CTC TAC ATC      1459
Ser Tyr Val Asn Ser Asn Met Glu Ser Ala Val Gly Ser Leu Tyr Ile
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AAG CGG GCC TTC TCC AAG GAC AGC AAG AGC ATA GTC AGT GAG CTT ATC      1507
Lys Arg Ala Phe Ser Lys Asp Ser Lys Ser Ile Val Ser Glu Leu Ile
            455                 460                 465

GAG AAG ATA CGG TCC GTG TTT GTG GAT AAC CTG GAC GAG TTG AAC TGG      1555
Glu Lys Ile Arg Ser Val Phe Val Asp Asn Leu Asp Glu Leu Asn Trp
        470                 475                 480

ATG GAT GAG GAA TCC AAG AAA AAG GCC CAG GAA AAG GCC TTG AAT ATC      1603
Met Asp Glu Glu Ser Lys Lys Lys Ala Gln Glu Lys Ala Leu Asn Ile
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CGG GAA CAG ATC GGC TAC CCT GAC TAC ATT TTG GAA GAC AAT AAC AGA      1651
Arg Glu Gln Ile Gly Tyr Pro Asp Tyr Ile Leu Glu Asp Asn Asn Arg
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CAC CTG GAT GAG GAA TAC TCC AGT CTG ACT TTC TCA GAG GAC CTG TAT      1699
His Leu Asp Glu Glu Tyr Ser Ser Leu Thr Phe Ser Glu Asp Leu Tyr
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TTT GAG AAC GGG CTT CAG AAC CTC AAG AAC AAT GCC CAA AGG AGC CTC      1747
Phe Glu Asn Gly Leu Gln Asn Leu Lys Asn Asn Ala Gln Arg Ser Leu
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AAG AAA CTT CGG GAA AAG GTG GAC CAG AAT CTC TGG ATC ATT GGG GCT      1795
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        550                 555                 560

GCA GTG GTC AAT GCA TTC TAC TCC CCA AAC AGA AAC CTG ATC GTC TTT      1843
Ala Val Val Asn Ala Phe Tyr Ser Pro Asn Arg Asn Leu Ile Val Phe
    565                 570                 575

CCA GCG GGG ATC CTC CAG CCA CCC TTC TTC AGC AAG GAC CAA CCA CAG      1891
Pro Ala Gly Ile Leu Gln Pro Pro Phe Phe Ser Lys Asp Gln Pro Gln
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GCC TTG AAT TTC GGG GGC ATC GGG ATG GTG ATT GGA CAC GAG ATC ACA      1939
Ala Leu Asn Phe Gly Gly Ile Gly Met Val Ile Gly His Glu Ile Thr
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CAC GGC TTT GAT GAT AAC GGT CGG AAC TTT GAC AAG AAT GGC AAC ATG      1987
His Gly Phe Asp Asp Asn Gly Arg Asn Phe Asp Lys Asn Gly Asn Met
            615                 620                 625

CTG GAC TGG TGG AGC AAC TTC TCG GCC CGG CAC TTC CGA CAG CAG TCA      2035
Leu Asp Trp Trp Ser Asn Phe Ser Ala Arg His Phe Arg Gln Gln Ser
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CAG TGT ATG ATT TAT CAG TAC AGC AAC TTC TCT TGG GAA CTA GCA GAC      2083
Gln Cys Met Ile Tyr Gln Tyr Ser Asn Phe Ser Trp Glu Leu Ala Asp
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AAC CAG AAT GTG AAC GGA TTC AGC ACC CTC GGG GAG AAC ATC GCC GAC      2131
Asn Gln Asn Val Asn Gly Phe Ser Thr Leu Gly Glu Asn Ile Ala Asp
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AAC GGC GGT GTG CGG CAG GCA TAC AAG GCT TAC CTA CAG TGG CTA GCT      2179
Asn Gly Gly Val Arg Gln Ala Tyr Lys Ala Tyr Leu Gln Trp Leu Ala
                680                 685                 690

GAA GGC GGC AGA GAC CAG AGA CTG CCG GGA CTG AAC CTG ACC TAT GCT      2227
Glu Gly Gly Arg Asp Gln Arg Leu Pro Gly Leu Asn Leu Thr Tyr Ala
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CAG CTT TTC TTC ATT AAC TAT GCC CAG GTG TGG TGT GGG TCC TAC AGG      2275
Gln Leu Phe Phe Ile Asn Tyr Ala Gln Val Trp Cys Gly Ser Tyr Arg
        710                 715                 720

CCG GAG TTC GCC ATC CAG TCC ATC AAG ACA GAT GTC CAC AGT CCT CTT      2323
Pro Glu Phe Ala Ile Gln Ser Ile Lys Thr Asp Val His Ser Pro Leu
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AAG TAC AGG GTG CTG GGC TCA CTA CAG AAC CTA CCA GGC TTC TCT GAG      2371
Lys Tyr Arg Val Leu Gly Ser Leu Gln Asn Leu Pro Gly Phe Ser Glu
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GCG TTC CAC TGC CCA CGA GGC AGC CCC ATG CAC CCT ATG AAT CGA TGT      2419
Ala Phe His Cys Pro Arg Gly Ser Pro Met His Pro Met Asn Arg Cys
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GATGAGTGGT GGTGCCTGGT CCTGCGCCTT TTCCGGCCAG TGAGGGTCAG CGGCCCGGTA 2648

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Ser Ile Gly Lys Gln Leu Pro Leu Leu Asn Ser Leu Leu His Val Ser
50 55 60

Arg His Glu Arg Thr Val Val Lys Arg Val Leu Arg Asp Ser Ser Gln
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Lys Ser Asp Ile Cys Thr Thr Pro Ser Cys Val Ile Ala Ala Ala Arg
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Ile Leu Gln Asn Met Asp Gln Ser Lys Lys Pro Cys Asp Asn Phe Tyr
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Gln Tyr Ala Cys Gly Gly Trp Leu Arg His His Val Ile Pro Glu Thr
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130 135 140

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Ile Glu Lys Arg Asp Ser Glu Pro Leu Leu Asn Val Leu Asp Met Ile

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    210                 215                 220

Arg Arg Val Leu Ile Asp Leu Phe Ile Trp Asn Asp Asp Gln Asn Ser
225                 230                 235                 240

Ser Arg His Val Ile Tyr Ile Asp Gln Pro Thr Leu Gly Met Pro Ser
                245                 250                 255

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His Asp Val Thr Ala Leu Tyr His Arg Met Gly Leu Glu Glu Leu Gln
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Glu Arg Phe Gly Leu Lys Gly Phe Asn Trp Thr Leu Phe Ile Gln Asn
            340                 345                 350

Val Leu Ser Ser Val Gln Val Glu Leu Leu Pro Asn Glu Glu Val Val
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Val Tyr Gly Ile Pro Tyr Leu Glu Asn Leu Glu Glu Ile Ile Asp Val
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Asp Arg Ile Gly Ser Leu Ser Gln Arg Phe Lys Glu Ala Arg Val Asp
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Tyr Arg Lys Ala Leu Tyr Gly Thr Thr Met Glu Glu Val Arg Trp Arg
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Arg Ser Leu Lys Lys Leu Arg Glu Lys Val Asp Gln Asn Leu Trp Ile
545 550 555 560

Ile Gly Ala Ala Val Val Asn Ala Phe Tyr Ser Pro Asn Arg Asn Leu
565 570 575

Ile Val Phe Pro Ala Gly Ile Leu Gln Pro Pro Phe Phe Ser Lys Asp
580 585 590

Gln Pro Gln Ala Leu Asn Phe Gly Gly Ile Gly Met Val Ile Gly His
595 600 605

Glu Ile Thr His Gly Phe Asp Asp Asn Gly Arg Asn Phe Asp Lys Asn
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Gly Asn Met Leu Asp Trp Trp Ser Asn Phe Ser Ala Arg His Phe Arg
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Gln Gln Ser Gln Cys Met Ile Tyr Gln Tyr Ser Asn Phe Ser Trp Glu
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Leu Ala Asp Asn Gln Asn Val Asn Gly Phe Ser Thr Leu Gly Glu Asn
660 665 670

Ile Ala Asp Asn Gly Gly Val Arg Gln Ala Tyr Lys Ala Tyr Leu Gln
675 680 685

Trp Leu Ala Glu Gly Gly Arg Asp Gln Arg Leu Pro Gly Leu Asn Leu
690 695 700

Thr Tyr Ala Gln Leu Phe Phe Ile Asn Tyr Ala Gln Val Trp Cys Gly
705 710 715 720

Ser Tyr Arg Pro Glu Phe Ala Ile Gln Ser Ile Lys Thr Asp Val His
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Ser Pro Leu Lys Tyr Arg Val Leu Gly Ser Leu Gln Asn Leu Pro Gly
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Cys Gly Gly Trp Leu Arg His His Val Ile Pro Glu Thr Asn Ser Arg
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Tyr Ser Val Phe Asp Ile Leu Arg Asp Glu Leu Glu Val Ile Leu Lys
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gcc aag aca ctg tac cgc tcc tgc atg aac cag agt gtg ata gag aag       643
Ala Lys Thr Leu Tyr Arg Ser Cys Met Asn Gln Ser Val Ile Glu Lys
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aga gac tct gag ccc ctg ctg aac gtc tta gat atg ata gga ggt tgg       691
Arg Asp Ser Glu Pro Leu Leu Asn Val Leu Asp Met Ile Gly Gly Trp
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Pro Val Ala Met Asp Lys Trp Asn Glu Thr Met Gly Pro Lys Trp Glu
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gtc atc tac ata gac cag ccc acc ttg ggc atg ccc tcc cgg gag tac       883
Val Ile Tyr Ile Asp Gln Pro Thr Leu Gly Met Pro Ser Arg Glu Tyr
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tat ttc aag gaa gac agc cac cgg gta cgg gaa gcc tac ctg cag ttc       931
Tyr Phe Lys Glu Asp Ser His Arg Val Arg Glu Ala Tyr Leu Gln Phe
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atg aca tca gtg gcc act atg ctg agg aga gac ctg aac ctg ccc ggg       979
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Thr Ala Leu Tyr His Arg Met Gly Leu Glu Glu Leu Gln Glu Arg Phe
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tct gtg caa gtt gag ctg ctc ccg aat gag gag gtg gtg gtc tat ggc      1219
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Ser Tyr Val Asn Ser Asn Met Glu Ser Ala Val Gly Ser Leu Tyr Ile
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gag aag ata cgg tcc gtg ttt gtg gat aac ctg gac gag ttg aac tgg      1555
Glu Lys Ile Arg Ser Val Phe Val Asp Asn Leu Asp Glu Leu Asn Trp
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atg gat gag gaa tcc aag aaa aag gcc cag gaa aag gcc ttg aat atc      1603
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cgg gaa cag atc ggc tac cct gac tac att ttg gaa gac aat aac aga      1651
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cac ctg gat gag gaa tac tcc agt ctg act ttc tca gag gac ctg tat      1699
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aag aaa ctt cgg gaa aag gtg gac cag aat ctc tgg atc att ggg gct      1795
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gca gtg gtc aat gca ttc tac tcc cca aac aga aac ctg atc gtc ttt      1843
Ala Val Val Asn Ala Phe Tyr Ser Pro Asn Arg Asn Leu Ile Val Phe
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cca gcg ggg atc ctc cag cca ccc ttc ttc agc aag gac caa cca cag      1891
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gcc ttg aat ttc ggg ggc atc ggg atg gtg att gga cac gag atc aca      1939
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cac ggc ttt gat gat aac ggt cgg aac ttt gac aag aat ggc aac atg      1987
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ctg gac tgg tgg agc aac ttc tcg gcc cgg cac ttc cga cag cag tca      2035
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aac cag aat gtg aac gga ttc agc acc ctc ggg gag aac atc gcc gac      2131
Asn Gln Asn Val Asn Gly Phe Ser Thr Leu Gly Glu Asn Ile Ala Asp
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aac ggc ggt gtg cgg cag gca tac aag gct tac cta cag tgg cta gct      2179
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cag ctt ttc ttc att aac tat gcc cag gtg tgg tgt ggg tcc tac agg      2275
Gln Leu Phe Phe Ile Asn Tyr Ala Gln Val Trp Cys Gly Ser Tyr Arg
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ccg gag ttc gcc atc cag tcc atc aag aca gat gtc cac agt cct ctt      2323
Pro Glu Phe Ala Ile Gln Ser Ile Lys Thr Asp Val His Ser Pro Leu
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Gln Tyr Ala Cys Gly Gly Trp Leu Arg His His Val Ile Pro Glu Thr
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Asn Ser Arg Tyr Ser Val Phe Asp Ile Leu Arg Asp Glu Leu Glu Val
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Ser Arg His Val Ile Tyr Ile Asp Gln Pro Thr Leu Gly Met Pro Ser
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Val Leu Ser Ser Val Gln Val Glu Leu Leu Pro Asn Glu Glu Val Val
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Ser Tyr Arg Pro Glu Phe Ala Ile Gln Ser Ile Lys Thr Asp Val His
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Ser Pro Leu Lys Tyr Arg Val Leu Gly Ser Leu Gln Asn Leu Pro Gly
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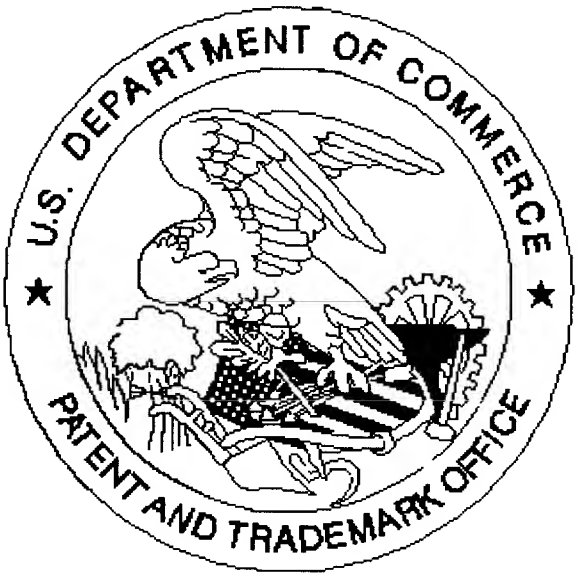

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